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Rh_o FACTOR IN JAPANESE. ITS RELATION TO TRANSFUSION REACTIONS.

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The Rh_o(D) factor is the most antigenic of all Rh-Hr factors and, threfore, the most important clinically. Thus, for clinical purposes, it suffices to make the tests with use of anti-Rh_o serum only. This procedure is known as Rh testing, by which individuals may be classified as either Rh_o-positive or Rh_o-negative (Rh-positive or Rh-negative simply indicated means Rh_o-positive or Rh_o-negative, respectively). Rh_o-negative individuals may be sensitized by transfusion of Rh_o-positive blood or by pregnancies of Rh_o-positive fetus, resulting in formation of antibodies against the Rh_o factors, which are frequently responsible for occurrence of transfusion reactions or erythroblastosis foetalis. Therefore, a Rh_o-negative individual needs clinically special cares. While the incidence of Rh_o-negative individuals in Caucasians is approximately 15 per cent, that in Japanese is estimated to be some 1/10 of the percentage in the former. Consequently, the problems in connection with the Rh_o factor in Japanese have been disregarded. However, the number of the samples studied, from which the incidence of the Rh_o-negative individuals in Japanese has been calculated, is not large enough so that it is imperative to study a larger number of samples in order to draw out a definite conclusion. Moreover, it seems to be of urgent importance to clarify whether the Rh_o factors, in case of blood transfusions among Japanese, are actually negligible or not, since we, surgeons, usually make frequent blood transfusions. Therefore, a survey has been carried out in those cases, to which blood transfusions had previously been given, and some possible relationship between the transfusion reactions and the Rh_o factor has been analyzed.

I. A New Rh Testing Method.

Two types of antibodies against Rh_o antigen have been known: namely, agglutinins (bivalent, complete or agglutinating antibodies) and blocking antibodies

(univalent, incomplete or conglutinating antibodies). Serum containing bivalent anti-Rh_o antibodies is capable of producing visible clumping of erythrocytes both in saline media (saline test or agglutination reaction[33]) and in plasma or serum media, when it is mixed with the Rh_o-positive red blood cells. When the sera containing univalent anti-Rh_o antibodies (blocking antibodies) are used in saline media, on the other hand, they only "coat" the Rh_o-positive red cells and do not cause the visible clumping, but in media of protein solution of above a certain concentration, such as serum, plasma or albumin solution, visible clumping of cells will ensue (albumin test[11] or conglutination reaction[31,33]). Therefore, the technique shall be in some measure different according to whether agglutinins or blocking antibodies are used for Rh testing.

For Rh typing test, several methods are available: test tube method (WIENER[20,31,33]), slide method (DIAMOND & ABELSON[9,10] or DIAMOND & DENTON[11]) and capillary tube method (CHOWN[3,5]). The capillary tube method is the most economical thereamong, but the reading with this method is not so clearcut that false positive or false negative results may readily occur. Besides, only bivalent antibodies can be used in the capillary tube method.

Having modified the CHOWN's capillary tube method, we suggest that the following new method shall advantageously be used, which seems to cover the drawbacks and have the merit of the capillary method.

1. MATERIALS AND APPARATUS.

(a) Capillary tubes : They are approximately 10cm in length, having the inside diameter of 0.5 mm, and are kept aseptic. Such tubes can easily be made with some practice.

(b) Anti-Rh_o serum : Serum containing anti-Rh_o blocking antibodies of such a high potency must be used that its agglutination titer measured by the test tube method with use of the standard Rh_o-positive red cells of median value of agglutinability is 32 or more.

(c) Bloods to be tested : They should be fresh and treated with an anticoagulant.

(d) Agglutination stand: Any home-made stand is available. What is needed is to hold the capillary tubes at an inclination of 45 degrees against the horizontal.

(e) A small gas flame, (f) slides, and (g) a file.

2. TECHIQUES AND READING.

As in case of the Chown's method, the tip of a capillary is dipped in the antiserum to allow a column of the serum to run in, approximately 2 cm. Then dip this end of the capillary into the blood to be tested, allowing a column of the blood to run in, about 1 cm. Blow the content of the capillary out on a clean glass slide and mix them. Thereafter, let the mixture run back into the middle portion of the capillary lumen. Seal its one end by melting the tip with use of a small gas flame, and then set it, inclined about 45 degrees to the horizontal, on the stand. Keep it in the incubator at the temperature of 37° C for 30

minutes.

Reading : The technique above-described is essentially not different from the CHOWN's method, in which the reading is done by observing the capillary containing the mixture of the serum and the red cells to be tested. But, in our new test method, the mixture is gently blown out of the capillary after cutting off ca. 1 cm of the sealed end by a file, on a slide in form of a streak, 1 to 2 cm in length, and the reading will then be made immediately, with naked eyes, before the mixture begins to dry.

Reactions : If the blood is Rh_o-positive, red cells gather themselves into one or several clumps and the remaining liquid becomes completely clear, free from suspended cells, as far as the antiserum is in possession of sufficient potency. When the blood is Rh_o-negative, on the other hand, clumping will never be observed but the mixture constantly shows homogeneous turbidity due to suspension of the cells. In other words, the reading in our method is quite clearcut and the results obtained are very reliable so far as the serum used is of sufficient potency.

3. SOME MINOR REMARKS.

(a) When the serum used is weak in potency, false negative results will often be obtained.

(b) If the inside diameter of a capillary tube is too small, the procedures of testing would be hard or even impossible to be carried out precisely. Since the clumps in the mixture are readily broken into small fragments if they are blown out through a capillary with a too small calibre, the consequent reading may tend to be judged as negative (false negative). Furthermore, when a too small calibered capillary tube is used, the mixture will be too small in amount and lead to the difficulty in recognizing the clumps at a glance, as well as to the prompt drying of the liquid, thus resulting in a false reading. Therefore, a capillary with a bore of more than 0.5 mm up to 1 mm is preferable for the beginners to use.

(c) Bloods to be tested should always be fresh and prevented from coagulation. For the latter purpose, the dried anti-coagulant may be added. Either 3.8 per cent solution of sodium citrate which is generally in use for determination of E. S. R. or 4 per cent citrate solution used for blood transfusion can also be availed, as anticoagulant, in an amount of 1/4 and 1/10, respectively, of the total volume. DIAMOND & DENTON[11] reported that the agglutination which took place in plasma media with use of blocking antibodies was weakened or interrupted when 1/3 volume of saline was contained in the media. However, the amount of water in the anticoagulants stated above is too small to give any influence on the results of the testing and, accordingly, there may be no probability that the blocking antibodies of the serum fail to give rise to distinguishable clumps in testing the Rh_o-positive red cells.

(d) As the Rh_o factor is relatively more labile than the AB substance,[16,22,25] it has been recommended to test fresh blood only. POTTER[26] said that the blood mixed with anticoagulant and kept in a refrigerator would ordinarily remain in a satis-

factory condition for testing for five to seven days. And cells in an undisturbed clot might be usable for two weeks or longer provided they had been under constant refrigeration. HATTERSLEY & FAWCETT[15] and WALL et al[30] reported that bloods preserved in ALSEVER's or ACD-solution held their agglutinability and specificity for a fairly long time. However, our experiment of measuring the agglutinability of the preserved red cells of 5 Rh_o-positive adult Japanese, daily for three days, disclosed that there was a gradual reduction of the agglutinability in proportion with the elapse of time, even though the red cells were preserved in a refrigerator and mixed with a 1/10 volume of 4 per cent citrate solution, i. e. the sera, which were sufficient to clump the red cells on the first day, needed to be increased in amount for the following days, each day 2 to 4 folds than the day before. In avoidance of false negative results due to reduction of agglutinability fresh bloods must be used for testing.

(e) At the time when we blow out the mixture contained in a capillary and spread it on the slide for reading, it is necessary to take care not to make air bubbles which may make the reading difficult. For prevention of bubbling, it would be wise to place one end of the capillary softly on the slide and carefully blow the mixture out, at the same time moving slowly the tip of the capillary from one side to the other, the tip being constantly kept in contact with the slide.

4. Reliability of the New Test Method.

In an attempt to determine the reliability of the new test method, the result of examination with our new method has been compared with that with other Rh testing methods, such as the test tube method (WIENER[31]), the slide method (DIAMOND & ABELSON[9]) and the capillary tube method (CHOWN[3, 4]), respectively. In this experiment, the same two fold serial dilutions of the anti-Rh_o blocking serum against the same standard Rh_o-positive red cells were used. For dilution of the sera and preparation of the suspension of the red cells, the same human sera of Group AB were used throughout the procedures. The Rh_o-positive standard red cells were of O-Group and had the median value of agglutinability in Japanese. Although the CHOWN's original method is based upon the agglutination reaction, we have determined the conglutination reaction. The results are given in Table 1. The sera which gave a remarkable clumping of (++) or more are at a glance decisively judged as positive, whatever method may be used. In Rh testing, it is needless to say that the reading must be so clearcut that the judgement of positive or negative reactions can at a glance and without fail be established. In this regard, the new test method is inferior to the test tube method, but is evidently superior to the slide method and the capillary method.

5. COMMENT.

Although the method advocated by CHOWN is obviously simple and economical, the reading with this method is not always as clearcut as has been asserted (cf. 1, 8, and 12[10]). The drawback in CHOWN's method was pointed out by DIAMOND & ABELSON and KRIEGER & WEIDEN[19]. As we have unfortunately never had an op-

portunity to use the anti-Rh_o complete antibodies for this method, we are unable to criticize duely the CHOWN's original method. However, judging from our own experiences with CHOWN's technique, by which we made the ABO grouping with use of anti-A and -B sera and the Rh testing with use of anti-Rh_o blocking sera, it is presumed that the reading with CHOWN's method may not absolutely be clearcut. If reading is made by observation of the mixture being kept in the capillary, there seems to be little difference in the positive reactions according to whether the sera used are weak or more potent. Thus it is possible that positive reactions due to rouleaux formation may be obtained, while on the other hand despite an intense agglutination a false negative can be simulated, since the red cells are apt to adhere to the inner surface of the glass capillary and the clumps tend to be far smaller than they should be. With our new testing method, on the contrary, a judgement as to whether the reaction is positive or negative can clearly and unmistakably be decided even in those cases where the reading after CHOWN's method may be mistakable for a false reaction.

As already described, the titer of the sera to be used for this new method should be 32 or more as measured by the test tube method. The reason is as follows: To get a clearcut result by our method, the potency of the sera should have the agglutination titer of 8 or more, as measured by the test tube method, against the red cells to be tested (cf. Table 1). However, the agglutinability of

Table 1. Comparison of Sensitivity of Different Rh Testing Methods.

Anti-Rh_o Serum: No. of Test Tube	1	2	3	4	5	6	7	8	9	10
No. of Two Fold Serial Dilut.	1	2	4	8	16	32	64	128	256	Control
(a) Test Tube Method	⫲	⫲	⫲	⫩	⫩	+	−	−	−	−
(b) Slide Method	⫲	⫩	+	±	−	−	−	−	−	−
(c) Capillary Tube Method	⊕	+	+	+	+	+	±	−	−	−
(d) New Rh Testing Method	⫲	⫲	⫩	+	+	±	−	−	−	−

NB

(1) In (a), (b) and (d) :

⫲ : Clumping of the cells in one fragment without turbidity.
⫩ : That in a few fragments without turbidity.
+ : That in minor fragments with turbidity.
± : That in hardly visible fragments with turbidity.
− : Evidently turbid without visible clumping.

(2) In (c) :

⊕ : Clumping of the cells in an elongated clot.
+ : That in a beaded layer along the lower side of the capillary tube.
± : That in a beaded layer, hardly discernible.
− : No clumping, forming a smooth line along the lower side of the capillary tube.

the Rh_o-positive red cells against anti-Rh_o sera varies individually. In our study of 20 adult Japanese it was confirmed that the highest number of the two fold serial dilution of the same anti-Rh_o blocking sera sufficient to cause the agglutination against the red cells varied between $2^{-1} \times n$ and $2^2 \times n$, if "n" was taken

as the highest dilution number of the sera against the standard red cells of the median value of agglutinability. For the sake of reliability, the sera having a titer of $2^2 \times 8$ or more, as measured by the test tube method against the standard red cells, has to be used, if a clear positive result should be obtained by our method even against the red cells of the lowest agglutinability. Thus, the failure due to weak sera, which is, as pointed out by DIAMOND,[8] one of the important causes of Rh testing errors, can well be avoided.

For routine laboratory use the aseptic capillary tubes are prepared, in which the anti-Rh_o blocking human sera for slide test has previously been allowed to run in and both ends of which have been sealed by melting on a gas flame. These capillaries are preserved in a refrigerator. They are ready for use, if both ends sealed are cut off by a file. Roughly estimated, the number of the blood samples subjected to test with use of the same amount of the antisera, is, by our method, 20 times as large as that by the slide method.

For detection of Rh_o antibodies, BERLIN[1] carried out the conglutination reaction after the CHOWN's method. According to CHOWN & LEWIS[1] and DISCOMBE & MEYER,[13] however, it would be necessary to use the complete antibody serum in the media of lesser viscosity in order to obtain accurate results by CHOWN's method, although the blocking antibodies are generally more potent, more stabile and more easily available than the complete antibodies and, therefore, the former are more widely in use for Rh testing by slide test method than the latter.[8,13] In this connection, our test method, for which the blocking sera are usable, has another superiority to the CHOWN's method.

Since not saline but protein containing media are used in testing, our method is based on the conglutination reaction. However, the agglutinating antibodies in saline media can also be used for Rh testing by our method. Furthermore, the ABO grouping is also possible by this method, if the anti-A and -B sera are taken instead.

II. Rh_o Factor in Japanese.

As is shown in Table 2, the incidence of the Rh_o-negative individuals in Japanese has been estimated to be much lower than that in Caucasians, the former being around one tenth of the latter (approximately 15 per cent). However, the number of the samples studied in Japanese is not large enough. It seems, therefore, necessary to investigate larger samples before we definitely admit the percentage hitherto reported. On this account, Rh testing was carried out in a total of 3228 Japanese, both in Kyoto and in Osaka, with use of our method. The anti-Rh_o blocking human serum used was given by some kind investigators in the United States. The result obtained showed that only 18 Japanese out of 3228 were Rh_o-negative, the incidence being about 0.6 per cent. This will say that almost all Rh_o-negative Japanese will receive Rh_o-positive blood, whenever a blood transfusion is made on the basis of the ABO-grouping only. The consequence is that the Japanese recipient of Rh_o-negative blood type is much more frequently dispo-

Table 2. Percentage of Rh_o-negative Individuals in Japanese

Name of the Reporters	Total No. of Tested Individuals	% of Rh_o-neg.	Antisera Used for Typing (Testing Method)
Waller & Levine (1944)[29]	150	1.3	
Miller & Taguchi (1945)[24]	280	1.1	Anti-Rh_o Human Serum (Test Tube Method)
Crown et al (1946)[5]	217	0.5	Anti-Rh_o Blocking Serum (Capillary Tube Method)
Ogawa (1947)[41]	415	9.11	Antiserum of Guineapigs Immunized with Rhesus Red Cells (Slide Method ?)
Schneider & Hughes (1948)[27]	459	1.5	Anti-Rh_o Serum (Test Tube Method)
Ino (1948)[37]	620	3.1	Antiserum of Guineapigs Immunized with Rhesus Red Cells (Test Tube Method)
Kaku & Niimura (1948)[38]	1,011	1.3	Antiserum of Guineapigs Immunized with Rhesus Red Cells
Zeze (1951)[42]	482	1.66	Antiserum of Guineapigs Immunized with Rhesus Red Cells
Kamiya (1952)[39]	1,141	3.4	Antiserum of Guineapigs Immunized with Rhesus Red Cells
Yokoyama (1953)	3,228	0.6	Anti-Rh_o Blocking Human Serum for Slide Test (New Method)

sed to iso-immunity, due to the Rh_o-incompatibility, than in the case of Caucasians. And, since it is necessary to test a great number of Japanese in order to find out a compatible Rh_o-negative donor every time when a blood transfusion is done, it would be nearly impossible to get a suitable donor in an emergency unless such a system as blood banks are immediately available, where Rh_o-negative donors are listed by previous grouping.

III. Rh_o Factor in the Saliva.

Concerning the presence of the Rh_o factor in the saliva, like in case of AB sudstances, there have been diverse opinions. Wiener & Forer[37] and Levine & Katzin[21] stated that the Rh_o factor was entirely absent in the saliva as in case of MN substances, while Boorman & Dodd[2] reported that it was discovered in the saliva of the Rh_o-positive individuals, although in a very small amount. Witebsky & Mohn[36] demonstrated the Rh_o factor in the amniotic fluid of the Rh_o-positive fetuses quite independently of tne presence of AB substances in the fluid. In an attempt to determine whether the Rh_o factor is to be found in the saliva of Japanese or not, experiments have been carried out, in which the inhibitory effect of the saliva, mixed with the anti-Rh_o sera, on the agglutinating potency of the sera have been measured.

1. Materials and Methods.

On account of the conglutination reaction with use of anti-Rh_o blocking antibodies, the same human sera pooled from several individuals of Group AB were used to dilute the anti-Rh_o blocking antibodies and also to suspend the red cells throughout this experiment.

(a) Standard anti-Rh_o sera : Anti-Rh_o blocking human sera for the slide test diluted by the sera of Group AB ; the titer of which is 8 against the standard Rh_o-positive red cells when measured by the test tube method. (Add one drop of 10 per cent cell suspension to 0.4 cc of diluted sera; Keep 2 hours in the incubator of 37° C, then read!)

(b) Standard red cell suspension: 10 per cent suspension, in the sera of Group AB, of Rh_o-positive red cells of Group O with the median value of agglutinability.

(c) Saliva to be tested : Supernatant of fresh saliva, stirred and centrifuged, are diluted by AB sera quadruply.

(d) Determination of the Blood Groups and Types :

(1) ABO grouping : by usual slide method.

(2) Typing of secretor or nonsecretor a. m. KOSHINO.[40] (The precipitation with use of the fowl sera immunized with the boiled saliva of a secretor of Group A).

(3) Rh testing : by our new method.

(e) Two series of experiments were done:

In the first series, the salivas of 46 Japanese were tested. The mixture of the quadruple dilution of the saliva to be tested and the same amount of the standard sera was placed in an incubator at the temperature of 37° C for 2 hours and, then, its agglutination titer for red cells in two fold serial dilution by the test tube method was determined. (One drop of the standard red cell suspension was added to 0.4 cc of the diluted mixture and reading was made after the incubation at 37° C for 2 hours.)

(f) In the second series of the experment, the preciseness of the technique used in the first series was examined. In order to see the minimal limit of the Rh_o factor in the saliva, if ever detectable, the suspensions of the standard red cells, in place of the saliva, were taken in a concentration of 1, 2, ..., and 32 per cent, respectively. Each suspension was further diluted to 4 volumes, mixed with the same amount of the standard anti-Rh_o sera and incubated at the temperature of 37° C, for 2 hours. After removal of red cells by centrifugation, the aggiutination titer of the media was measured by the two fold serial dilution in the same way as in the first series.

2. RESULTS.

The results are given in Table 3. In the first series none of the salivas could evidently inhibit the agglutinating potency of the standard anti-Rh_o sera. But in the second series, it was shown that the minimal amount of the Rh_o factor in the saliva, capable of inhibiting the agglutination of the standard anti-Rh_o sera, must be equivalent to, or more than, that contained in the same volume of the

Table 3. Inhibitory Effect of Saliva on the Agglutinating Ability of Anti-Rh_o Serum.

	Objects Tested from Individuals of			Agglutination No. of Dilut. of the Mixtures (0.2cc of Anti-Rh_o Standard Serum and 0.2cc of Saliva in 4 Fold Dilut.)				
	ABO-Group.	Secretor(S) or Nonsecretor(s)	Rh-Test.	1	2	4	8	16
I Series : Saliva of only 11 of 46 Tested Individuals	O	S	+	##	+	±	−	−
	O	s	+	##				
	A	S	+	##	+	+	−	−
	A	s	+	+	+	+	−	−
	B	S	+	+	+	±	−	−
	B	s	+	##	##	+	±	−
	AB	S	+	+	+	+	−	−
	AB	s	+	##				
	A	S	−	+	±	±	−	−
	A	S	−	##	+	±	−	−
	B	s	−	##	+	±	−	−
II Series : Suspension of Standard Rh_o-Positive Red Cells	Its Concentration		1%	##	+	±	−	−
			2%	##	+	−	−	−
			4%	+	±	−	−	−
			8%	±	−	−	−	−
			16%	−	−	−	−	−
			32%	−	−	−	−	−
Control	Saline			##	+	±	−	−

4 per cent suspension of the standard red cells. In summarizing, it may be said that the inhibitory ability of the saliva against the standard sera is lower than that of the 4 per cent suspension of the standard red cells of the same volume.

3. COMMENT.

BOORMAN & DODD[2] was of the opinion that the Rh_o factor must be non-water-soluble, because it was widely distributed in the tissues but almost entirely absent in the bodily fluids. MOSKOWITZ et al,[25] however, reported that they succeeded in separating some water-soluble fraction containing Rh_o factor from the human red cells. From the results obtained in our experiments, it can hardly be concluded that the Rh_o factor is absolutely absent in the saliva. A definite conclusion must be awaited for until further studies have been accomplished. But, even the largest amount of the Rh_o factor in the saliva, if ever present, is supposed to be so negligibly small as less than 4/100 in quantity of the Rh_o factor con-

tained in the same volume of the standard red cells. This is significantly contrasted with the fact that AB substances are found in a relatively high concentration in the bodily fluids, for example 128～10124 in the saliva and 8～32 in the erythrocytes.[34]

Judging from what has been described adove, it seems possible to neutralize the iso-agglutinins, α and β, contained in the anti-Rh_o human sera, by mixing these together with the saliva of the secretor without reducing the anti-Rh_o potency of the sera, as WIENER[34] and DIAMOND,[8] etc., have asserted. Provided that the Rh_o factor in the bodily fluids other than the saliva, in the plasma in particular, is so small in amount as to be neglibible, or not at all present, it would not be absolutely necessary to use only the Rh_o-negative sera of Group AB as media for Rh_o conglutination reaction, although POTTER[26] remarked that it would be preferable to use the Rh_o-negative AB serum. According to KINDLEY,[18] no inhibition due to the neutralization of Anti-Rh_o antibodies of the sera ever occurred actually during his study on the allergy resulting from the Rh_o factor. As pointed out by LEVINE & KATZIN[21] aud BOORMAN & DODD,[2] it would be readily understood why the erythroblastosis foetalis due to the Rh_o-incompatibility between the mother and her fetus takes place more frequently than that due to the ABO-incompatibility, since the Rh_o factor is considered to be almost entirely absent in the bodily fluid, while ABO substances are abundantly present therein and are able to neutralize the antibodies.

IV. Transfusion Reactions in Japanese in Relation to the Rh_o Factor.

Out of 5386 blood transfusions, reported by DEGOWIN,[6] reactions were met with in 186 cases, in 6 of which reactions were caused by the iso-immunization due to the Rh_o-incompatibility. Thus, the incidences of transfusion reactions due to Rh_o-incompatibility were ca. 0.1 per cent of all the transfusions (5386 cases) and 3.2 per cent of all the transtusion reactions(186 cases). Also KILDUFFE & DEBAKEY[17] reported that hemolytic reactions occurred in about 0.18 per cent of 43, 284 persons who had received blood trausfusions. If the fact is taken into concideration that 80～90 per cent of the hemolytic reactions,[7,28] if present, in Caucasians are due to the Rh_o-incompatibility, it may be sumised that hemolytic reactions due to the Rh_o-incompatibility take place in around 0.1 per cent. As the incidence of Rh_o-negative individuals in Japanese is remarkably lower than that of Caucasians, it is presumable that the frequency of reactions due to Rh_o factor might be far less than that in Caucasians.

Regarding erythroblastosis foetalis in Japanese, several reports are available. However, as to the transfusion reactions due to the Rh_o factor in Japanese, I have been so far able to find only one report published by KAMIYA,[39] who presented 10 cases of transfusion reactions. Out of the 10 cases, only one proved to be Rh_o-negative and the remaining 9 Rh_o-positive.

Transfusion reactions in relation to Rh_o-factor have been studied in 159 of our

own cases :

1. MATERIALS AND METHODS.

The materials were largely consisted of those patients admitted to the University Hospital of Kyoto, who showed blood transfusion reactions (159 cases). Generally speaking, hemolysis is the main factor of the Rh_o-incompatible transfusion reactions. In few instances, however, urticariae may be the only clinical manifestation of the reactions due to Rh_o-incompatible transfusion.[6,18,31] It is, therefore, not justifiable to determine by mere clinical signs whether the reaction is due to a Rh_o-incompatible transfusion or not, and whether the reaction is of hemolytic nature or non-hemolytic, as has been discussed by FLINK.[10] Accordingly, all subjects with clinical signs relating more or less to the reactions were carefully examined. Classification of the reactions with corresponding data is listed in Table 4.

Table 4. Freguency of Clinical Signs of Transfusion Reactions

Clinical Signs	No.
Chills and Fever	154
Urticaria	105
Hemoglobinuria	8
Nausea and Vomiting	7
Pain in the Back of the Neck and Chest, and in the Lumbar Region	6
Anxiousness and Distress	5
Dyspnea and Palpitation	3
Flushes	2
Jaundice	2
Petechia	1
Singultus	1

2. RESULTS.

The results are shown in Table 5. None was Rh_o-negative among the 159 patients. ABO grouping errors were found in the records in 13 cases.

3. COMMENT.

It has been generally agreed that sensitization in the case of Caucasians does occur not in all but in approximately one half of those Rh_o-negative persons, to whom Rh_o-positive bloods were given. The phenomenon of sensitization may probably be influenced by the amount of the blood introduced, by the frequency and the interval of transfusions, and by the individual difference in the ability of producing antibodies.[32] In this regard, WIENER has postulated a genetic theory by which he explained the varying responses exhibited by different individuals. It is often said that in Japan the amount of the blood transfused and the frequency of transfusion are usually less than those in Europe and in the United States.

Supposing that in Japanese the intensity of sensitization induced by the Rh_o-incompatibility in the Rh_o-negative individuals may be equal to that in Caucasians, the probable occurrence of hemolytic reactions due to the Rh_o-incompatibility in the former will be about 1/30 of that in the latter, because the percentage of Rh_o-negative individuals in Japanese is about 0.6 only. In other words, the

Table 5. ABO Grouping and Rh Testing of the 159 Patients with Reactions.

Rh-Testing		ABO-Grouping		Errors Found in Admission Journals (13 of 159 Patients)		
No. of Pos.	No. of Neg.	Group	No.	Group Discribed	Blood Infused	Clinical Signs (n/m)*
159	0	A	50	O	O+	Urticaria (1/7)
				O	O	Chills and Fever (1/1)
				O	O	Chills and Fever (1/1)
		B	37	AB	AB+	Anxiousness, Chills, Fever, and Hemoglobinuria (1/1)
				O	O	Fever (30/37)
				O	OL+	Chills and Fever (3&4/11)
		AB	17	A	A	Urticaria (1/1)
				A	OL+	Chills and Fever (5,7&8/8)
				B		Chills and Fever (1/10)
				O	OL+	Chills, Fever and Hemoglobinuria (1/1)
		O	55	A	A+	Vomiting, Pain in the Neck, Chest and Lumbar Region, Chills and Fever (1/11)
				B	B+	Palpitation, Pain in the Chest, Chills and Fever (1/2)
				AB	O	Urticaria (1/1)

NB + Preserved Blood

* n : Appearance of the Reactions at the "n"th Transfusion.
m : Number of Transfusions.

hemolytic reactions caused by the Rh_o-incompatibility will very rarely be met with in Japan ; about one to 30,000 transfusions. Actually the 159 Japanese patients showing transfusion reactions were confirmed to be all Rh_o-positive and the iso-immunization due to the Rh_o factor was disproved as the cause of their reactions.

The errors of ABO grouping recorded in the admission journals proved by re-examination to be the results of overlooking of an antigen or antigens in the part of patients, presumably due to the use of weak antisera.

SUMMARY AND CONCLUSIONS

(1) Having modified the CHOWN's capillary tube method, the author has devised a new Rh testing method ; the difference in the procedures being that the mixture of the antisera with the blood to be tested is blown softly out of the capillary on the slide to read.

(2) Among the 3,228 Japanese tested by our new method, the percentage of Rh_o-negative individuals revealed to be around 0.6.

(3) The Rh_o factor was not found in the saliva of a total of 46 Japanese tested.

(4) Among the 159 Japanese who had n ore or less transfusion reactions, none proved to be Rh_o-negative.

(5) From the clinical viewpoint, in Japan, it would be of no significance whether Rh testing would be done or not in the blood before it is transfused, since the incidence of transfusion reactions due to the Rh_o-incompatibility in Japanese is next to none. For the special cases, in which transfusion reactions had ever been observed, it would be advisable for them to utilize, whenever a blood transfusion is necessary, the blood bank system, where Rh testing can be done for them and Rh_o-negative donors are immediately available.

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和文抄錄

日本人の Rh_0 因子

特に輸血副作用との関係

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(1) 毛細ガラス管内で抗 Rh_0 血清と被検血球との凝集反応を起させ，之をガラス板上に吹き出して判読する新 Rh 型判定法を考案した．此の新判定法は所要材料が少量で，特殊な器具及び技術を必要とせず，且判読は明確である．

(2) 新 Rh 型判定法により，3228名の Rh 型を検査したところ，日本人の Rh_0 陰性者の頻度は約 0.6 % であつた．

(3) 抗 Rh_0 遮断抗体人血清の Rh 陽性血球に対する凝集力が，唾液により阻止されるかどうかを，46名の日本人について検査したが，此の検査方法による成績では，唾液中には Rh_0 因子が含有されていると認むべき所見は得られなかつた．

(4) 輸血副作用を呈した159名の日本人患者の Rh型を検査したが，皆 Rh_0 陽性で，之等の患者に関するかぎり，Rh_0 因子による同種免疫が原因であると認められるものはなかつた．

(5) 日本人の Rh_0 陰性者の頻度は上記の如く極めて小であるから，輸血に際し毎常 Rh 型判定を行う事は労多くして効が少い．輸血後毎常副作用を呈する如き特殊な症例に限つて Rh 型の判定が必要であつて，それが Rh_0 陰性であると判明した時には，予め Rh 型既知の給血者を準備してある血液銀行制度を利用する事が望ましい．